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Notes on rust fungi in China 3. *Puccinia adenocauli* comb. nov. and its life cycle and new host

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Abstract—The heteroecious and macrocyclic life cycle of *Aecidium adenocauli* was proved with field observations and inoculation experiments in China. Its spermogonial and aecial stages were produced on *Adenocaulon himalaicum,* and uredinial and telial stages were produced on *Carex onoei,* which is a new host for this rust fungus. Uredinial and telial stages (teleomorph) of this rust fungus were formerly described as *Puccinia carici-adenocauli* from Japan. However, the current International Code of Nomenclature (ICN (Melbourne)) has abolished the distinction between "anamorph" names and "teleomorph" names, and we propose the new combination *Puccinia adenocauli*, based on *Ae. adenocauli* 1913, which now has priority over *P. carici-adenocauli* 1999. Its neotype and epitype are also designated.

Key words—*Asteraceae*, *Cyperaceae, Pucciniomycetes,* taxonomy, *Uredinales*

Introduction

Aecidium adenocauli has been reported to produce spermogonial and aecial stages (anamorph) on *Adenocaulon himalaicum* Edgew. (*Asteraceae*) from Japan, Korea, Russian Far East, and China (Sydow & Sydow 1913, Ito 1950, Hiratsuka 1963, Azbukina 1974, Zhuang 1990, Hiratsuka et al. 1992). However, Kakishima et al. (1999) showed with inoculation experiments that its uredinial and telial stages (teleomorph) were produced on *Carex hakonensis* Franch. & Sav. and *C. uda* Maxim. (*Cyperaceae*) in Japan and described this teleomorph as a new species, *Puccinia carici-adenocauli.* Azbukina (2005) also reported

this species on *Ad. himalaicum* and *C. uda* from the Russian Far East based on morphological observations.

During investigations of rust fungi in Jilin Province, northeast China, we found the aecial stage of a rust fungus on *Ad. himalaicum*. This rust fungus was morphologically similar to *Ae. adenocauli,* but its uredinial and telial stages had not been clarified in China. Therefore, we carried out field survey and inoculation experiments to determine life cycle of this rust fungus. We report here the results of inoculation experiments with this rust fungus and its morphology based on specimens collected in China. We also discuss the taxonomic treatment of this fungus according to the current International Code of Nomenclature for algae, fungi, and plants (ICN (Melbourne); McNeill et al. 2012) and propose the new combination *Puccinia adenocauli*.

Materials & methods

Field survey and inoculation experiments

An aecial stage on *Ad. himalaicum* was found in the forest floor at Hongyegu, Jiaohe, Jilin, Jilin Province, China (43°42′13″N 127°04′18″E, alt. 537m) in June 2015 during a survey of rust fungi. This forest mainly consists of species of *Betula, Populus*, and *Quercus* and is a typical deciduous broad-leaved forest in this area. Uredinial and telial stages of this rust fungus were suspected to occur on species of *Carex* because a heteroecious species, *P. carici-adenocauli,* alternating between species of *Adenocaulon* and *Carex,* had been reported from Japan (Kakishima et al. 1999) and the Russian Far East (Azbukina 2005). Therefore, we looked for rust fungi on *Carex* species in the area where the aecial stage on *Ad. himalaicum* had occurred and collected telial stages on *C. onoei* Franch. & Sav. and *C. ussuriensis* Kom. in September 2015 and May 2016. These telial stages were morphologically identified as belonging to *Puccinia* and were used for inoculations.

Inoculation experiments were carried out during May–June 2016 at Jilin Agricultural University, Changchun, Jilin Province. For basidiospore inoculations the leaves with telia collected on 13 May 2016 (Fig. 2B) were kept in water for 1–2 hours to induce teliospore germination. These leaves were cut into small pieces (ca. 5 mm^2) and placed on healthy leaves of *Ad. himalaicum,* which was transplanted from the same forest cited above and maintained in plastic pots at Jilin Agricultural University. No natural infection of the plant from fields was confirmed before inoculations. The inoculated plants were kept in a moist atmosphere in a plastic box in darkness at 15–20°C for three days and then transferred to a place near windows at 15–20°C for observations.

Aeciospores produced on *Ad. himalaicum* by basidiospore inoculations were then used as inocula. Aeciospores were dusted onto wet filter papers (about 3 mm^2) and these papers were then placed on young healthy leaves of *C. onoei* planted in plastic pots. The plants were kept in a plastic box under the same conditions as basidiospore inoculations, later transferred to a place near windows.

Morphological observations

Specimens collected in the field or obtained from inoculations were used for morphological observations. Specimens of *Puccinia carici-adenocauli* including the type specimen (TSH-R1693) deposited in the Mycological Herbarium of the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan (TSH), were borrowed for comparative morphology.

Light microscopy (LM) was used to examine morphological characters including the size and shape of sori and spores. Spores or thin-sections of sori from specimens were mounted in a drop of lactophenol solution on glass slides for LM. Approximately 50 spores from each specimen were randomly chosen and the length, width, and wall thickness of spores were measured using Leica LAS X software attached to a Leica DM2000 microscope (Leica, Germany).

The surface features of spores were examined by scanning electron microscopy (SEM). For SEM, sori and spores obtained from dry specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium using a Hitachi MC1000 Ion Sputter Coater and examined with a Hitachi SU8010 SEM operated at 5–7 kV.

Dry specimens used in the experiments were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU).

Results & discussion

Life cycle

Seven to ten days after inoculations with basidiospores from teliospores on *C. onoei,* small yellow spots of spermogonia appeared on the upper leaf surface of *Ad. himalaicum* (Fig. 1A). About 7 days later, cupulate aecia with aeciospores were produced on the lower leaf surface of the same leaves (Fig. 1B). No infection was observed on *Ad. himalaicum* in the inoculations with teliospores from *C. ussuriensis;* this suggests that the *C. ussuriensis* rust is probably a different species from the rust on *C. onoei*. About 12 days after inoculations with aeciospores from *Ad. himalaicum,* pale yellow spots appeared on *C. onoei* and then powdery uredinia were produced (Figs 2C, 2E). The inoculation results confirmed that the rust fungus producing spermogonial and aecial stages on *Ad. himalaicum* was conspecific with the rust fungus producing uredinial and telial stages on *C. onoei*.

Morphology & taxonomy

Morphological observations using LM and SEM of specimens obtained from the field and inoculations showed that spermogonial and aecial stages on *Ad. himalaicum* and uredinial and telial stages on *C. onoei* were identical with the morphology of *P. carici-adenocauli* (anamorph: *Ae. adenocauli*) described by Kakishima et al. (1999). Their identity was also confirmed by comparative

morphology with specimens from TSH. However, according to McNeill et al. (2012: Art. 59.1), since 1 Jan. 2013, the earliest legitimate holomorphic name of this species is *Ae. adenocauli*, and *P. carici-adenocauli* is a later synonym (Kakishima et al. 1999). Therefore, we propose a new name based on the earlier name. Sydow & Sydow (1913) described *Ae. adenocauli* based on the specimen on "*Ad. bicolor*" [a misdetermination of *Ad. himalaicum*] collected at Kuzumaki-machi, Iwate Prefecture, Japan on 6 July 1907. This specimen was not found in any herbarium, including B (Berlin, Germany), and it is suspected that the specimen was lost during World War II. Therefore, we designate a neotype specimen on *Ad. himalaicum* for this species and also an epitype specimen on *Carex onoei.* We also provide a description based on specimens obtained in China.

Puccinia adenocauli (Syd. & P. Syd.) Jing X. Ji & Kakish., **comb. nov.** Figs 1–3

MycoBank MB817618

≡ *Aecidium adenocauli* Syd. & P. Syd., Ann. Mycol. 11: 111, 1913.

Type: Stages 0, I on *Adenocaulon himalaicum,* Japan, Yamanashi Prefecture, Yamanakako-mura, 25 May 1983, leg. M. Kakishima (**Neotype designated here**, TSH-R1689). Stage III on *Carex onoei,* China, Jilin Province, Jilin, Jiaohe, 13 May 2016, leg. J.X. Ji & M. Kakishima (**Epitype designated here**, HMJAU8275).

= *Puccinia carici-adenocauli* Kakish., Yokoi & Y. Harada, Mycoscience 40: 506, 1999.

Spermogonia subepidermal (type 4 of Cummins & Hiratsuka 2003). Aecia subepidermal, erumpent, *Aecidium*-type with peridia and catenulate spores. Aeciospores catenulate, globose, obovoid or sometimes angular, 13–18 × 10–15 µm (av. 15 × 12 µm), walls hyaline, 0.6 µm thick, verrucose with large granules. Uredinia subepidermal, pale yellow, erumpent, without paraphyses. Urediniospores pedicellate, obovoid or ellipsoid, 21–27 × 16–22 µm (av. 23 × 20 µm), walls hyaline, echinulate, 1.6–1.2 µm (av. 0.9 µm), germ pores obscure. Telia subepidermal, erumpent, dark brown to black. Teliospores 2-celled by transverse septum, borne singly on pedicels, clavate to oblong with round to obtuse apex and attenuate toward bases, constricted at septum, 30–47 × 15–26 µm (av. 40 × 20 µm), walls dark brown, smooth, 0.6–2.0 µm (av. 1.2 µm) thick, thickened at apex.

Specimens examined — **CHINA, Jilin Province:** Stages 0, I on *Adenocaulon himalaicum*, (Jilin, 24 June 2015, HMJAU8205, cultured at Jilin Agricultural Univ., Changchun, 11 June 2016, HMJAU8277, cultured at Jilin Agricultural Univ., Changchun, 24 June 2016, HMJAU8278, Tonghua, 8 June 2016, HMJAU8276); Stage II on *Carex onoei*, 2 July 2016, cultured at Jilin Agricultural Univ. (HMJAU8274); Stage III on *C. onoei*,13 May 2016, Jilin (HMJAU8275).

Hosts & distribution — Stages 0, I on *Adenocaulon himalaicum:* China (Zhuang 1990), Korea (Ito 1950, Hiratsuka 1963, Hiratsuka et al. 1992, Kakishima et al. 1999), Russian Far East (Azbukina 1974, 2005, Kakishima et al. 1999), Japan (Ito 1950,

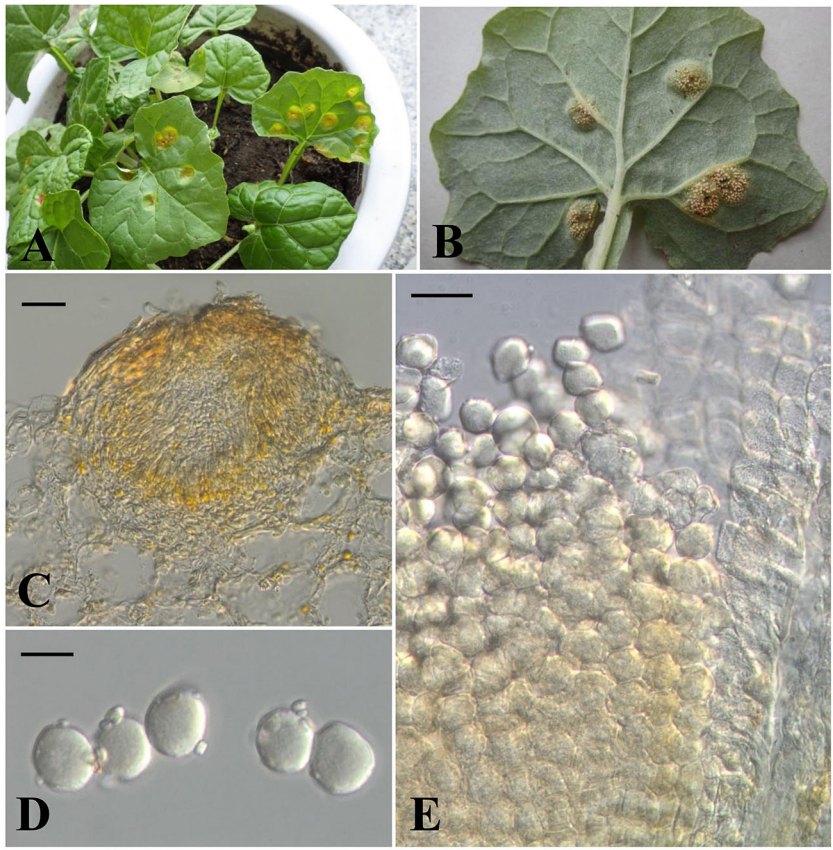

Fig. 1. *Puccinia adenocauli* on *Adenocaulon himalaicum*: spermogonial and aecial stages. A. Spermogonia on upper leaf surfaces produced by basidiospore inoculation. B. Aecia on lower leaf surface produced by basidiospore inoculation. C. Vertical section of a spermogonium. D. Aeciospores with large granules. E. Catenulate aeciospores surrounded by peridium in a vertical section of an aecium. Scale bars: C, D = 15 µm; E = 20 µm.

Hiratsuka et al. 1992, Kakishima et al. 1999). Stages II, III on *Carex hakonensis:* Japan (Kakishima et al. 1999). II, III on *C. uda:* Russian Far East (Azbukina 2005), Japan (Kakishima et al. 1999). Stages II, III on *C. onoei:* China.

The frequent occurrence of aecial stage on *Ad. himalaicum* indicates that this rust fungus is widely distributed in East Asia, although its uredinial and telial stages on *Carex* species have been rarely reported. Collection of this rust fungus on *Carex* is difficult because *Carex* species are short and their leaves are very

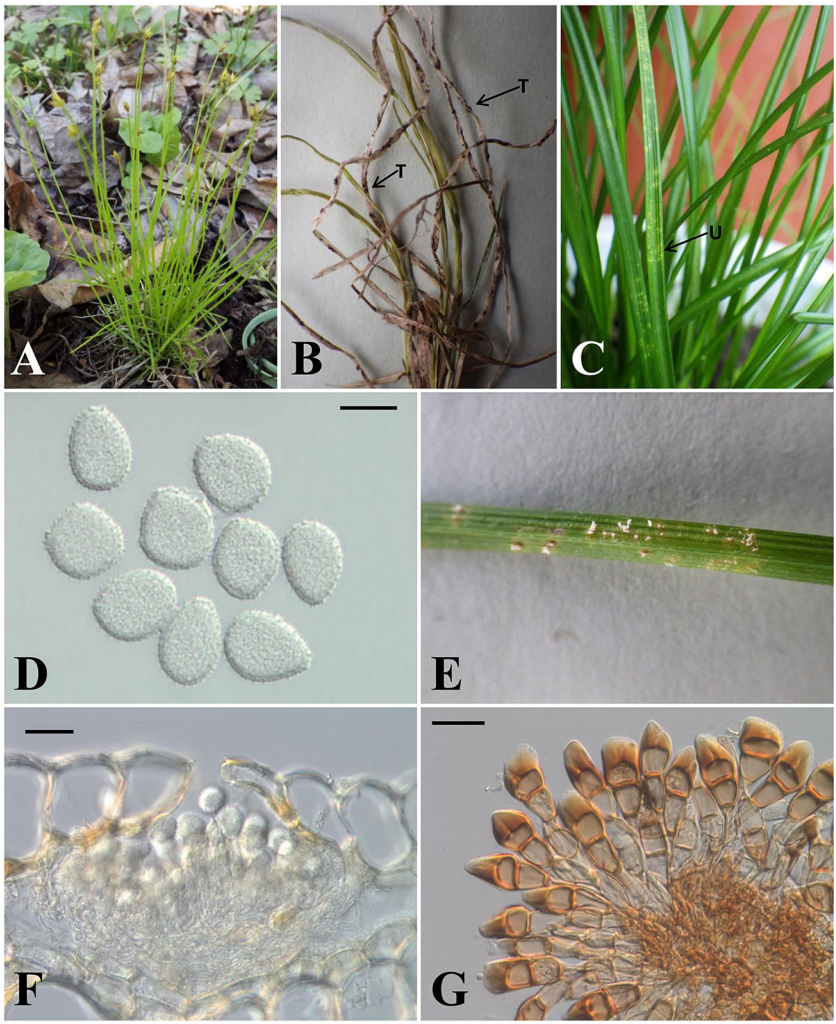

Fig. 2. *Puccinia adenocauli* on Carex *onoei*: uredinial and telial stages. A. *Carex onoei* with flowers taken on forest floor at Jilin, Jilin Province in May 2016. B. Dark brown telia (T) on the dead leaves. C. Uredinia (U) on the leaf surface produced by aeciospore inoculation. D. Echinulate urediniospores obtained by aeciospore inoculation. E. Pale yellow uredinia on lower leaf surface produced by aeciospore inoculation. F. Vertical section of a uredinium. G. Teliospores. Scale bars: D = 20 μm; F = 50 μm; G = 30 μm.

thin. Our field observations and inoculations have shown, for the first time, that *C. onoei* serves as uredinial and telial host of this rust fungus in China.

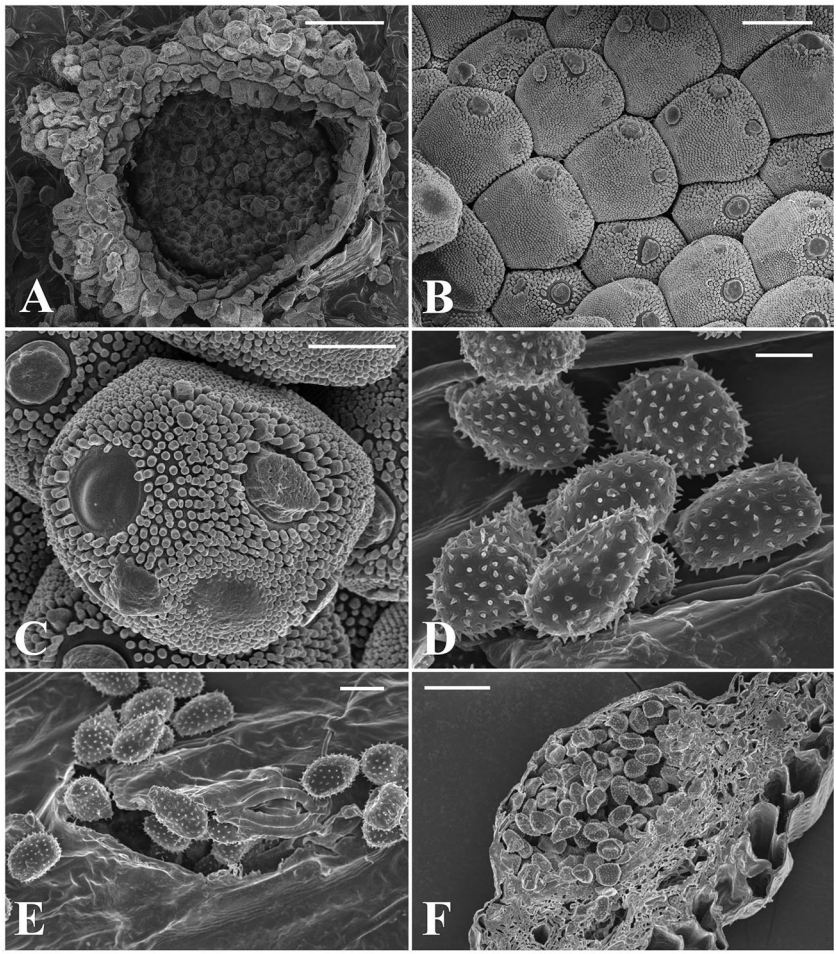

Fig. 3. *Puccinia adenocauli* observed with SEM. A. Aecium. B. Catenulate aeciospores in an aecium on *Adenocaulon himalaicum*. C. Verrucose aeciospore with large granules. D. Echinulate urediniospores. E. Uredinium produced on the leaf surface of *Carex onoei.* F. Vertical section of a uredinium. Scale bars: A = 40 μm; B = 10 μm; C = 5 μm, D = 15 μm; E = 20 μm; F = 50 μm.

Carex onoei also represents a new host for this rust fungus. Two other *Carex* spp., which are uredinial and telial hosts of this rust, have been reported from Japan and the Russian Far East (Kakishima et al. 1999, Azbukina 2005). These three *Carex* species (*C. hakonensis*, *C. onoei*, *C. uda*) belong to *Carex* sect. *Rarae* and it is, therefore, probable/possible that this rust fungus may be host specific to this section of *Carex* for its survival.

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